EFFECT OF LOMUSTINE ON N-ACETYLTRANSFERASE ACTIVITY IN RAT GLIOMA IN VITRO CULTURED CELLS

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Purpose : Arylamine N-acetylation capacity by the N-acetyltransferase (NAT) may be an important causative factor in the initiation of cancer. Lomustine is a commercially available nitrosourea derivative and chloroethyl nitrosourea compound which applied in antineoplastic therapy especially in central nervous system. The affect of lomustine on the activity of NAT in rat glioma in vitro cultured cells were investigated.

Materials and Methods : Rat glioma in vitro cultured cell cytosol (n=12) were used to investigate for the effect of lomustine on the activity of NAT toward 2-amniofluorene (AF) and p-aminiobenzoic acid (PABA) using an acetyl coenzyme A (AcCoA) recycling assay and high pressure liquid chromatography (HPLC).

Results : Activity of N-acetyltransferase in rat glioma in vitro cultured cell cytosol is found to be inhibited by lomustine. Its presence in the assay medium shows an inhibition in aminofluorene-N-acetyltransferase (AF-NAT) activity about of 17.6%, 31.5% and 45.4% in rat glioma in vitro cultured cells at 0.8 μM, 8 μM and 80 μM of lomustine concentrations respectively; while as p-aminobenzoic acid-N-acetyltransferase (PABA-NAT) activity decreased about 10.4%, 27.1% and 45.8% in rat glioma cells with 0.8 μM, 8 μM and 80(M of lomustine concentrations respectively.

Conclusion : The results of this study show that the NAT activity of rat glioma cultured cells are decreased by lomustine.

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Key words: Arylamine, N-acetyltransferase, Glioma, Lomustine

INTRODUCTION

The arylamines represent one of the documented classes of chemicals known to induce tumor in human [13,20]. N-acetylation is a major metabolic pathway of arylamine, and has been associated with carcinogen bioactivation. The reaction is catalyzed by cytosolic arylamine N-acetyltransferase (NAT), which required acetyl coenzyme A as a cofactor [21]. Acetyl coenzyme A-dependent arylamine NAT has been found in the tissues of several mammalian species [9,10,19] and in rat glioma cells [11]. Most of arylamine carcinogens, after undergo-

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ing metabolic activation, have been demonstrated to react with cellular DNA to form covalent DNA adducts, induce mutation and cause cancer in human [6,15,16]. Lomustine is commercially available nitrosourea derivative and chloroethyl nitrosourea compound which applied in anti-neoplastic therapy especially in central nervous system. In this study, the effect of lomustine on the activity of NAT in rat glioma cells were investigated.

MATERIALS AND METHODS

Chemicals and Reagents

Ethylenediaminetetraacetic acid (EDTA), 2-aminofluorene (AF), N-acetyl-2-aminofluorene (AAF), p-aminobenzoic acid (PABA), N-acetyl-p-aminobenzoic acid (N-Ac-PABA), acetylcarnitine, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), Tris, dimethyl sulfoxide (DMSO), dithiothreitol (DTT), Acetyl-Coenzyme A (AcCoA), and carnitine acetyltransferase were obtained from Sigma Chemical Co. (St. Louis, MO). All of the chemicals were reagent grade.

Rat glioma cell line

Rat glioma in vitro cultured cells were obtained from the cell bank of Veterans General Hospital-Taipei (Taiwan). The cultured cells were placed into 75 cm^2 tissue culture flasks (Gibco BRL, Grand Island, NY) and grown at 37°C under a humidified 5% CO2 atmosphere incubator in RPMI 1640 medium (Sigma Chemical Company, St. Louis, MO) supplemented with 10% fetal bovine serum (Gibco BRL, Grand Island, NY), 2% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin).

Preparation of rat glioma in vitro cultured cell cytosols

About 5×10^7 cultured cells were placed in 2 ml of the lysis buffer (20 mM Tris/HCl, pH 7.5 (at 4°C), 1 mM DTT, 1 mM EDTA, 50 mM PMSF, and 10 mM leupeptin). The suspensions were centrifuged at 9,000g for 1 min in a model 3200 Eppendorf/Brinkman centrifuge, and the supernatant fraction was subsequently centrifuged at 100,000g for 60 min. The supernatant was kept on ice for determinations.

Effect of lomustine on NAT activity in glioma cell cytosol

AcCoA-dependent N-acetylation of aminofluorene (AF) and p-aminobenzoic acid (PABA) were determined as described by Hung et al [11]. The mixture in the assay system consisted of 90 μL cytosol, 50 μL buffer solution (20 mM Tris/HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 1 mM acetyl carnitine), AF or PABA as the substrates, and 10 μL of various concentration of lomustine (0.8 μM, 8 μM and 80 μM), adjusted to pH 7.5, and incubated at 37°C for 10 min (n=12). The reaction was initiated by the addition of 20 μL AcCoA. The control was supplemented with 20 μL distilled water. The final concentration of AF or PABA was 0.1 mM, while that of AcCoA was 0.5 mM. The reaction mixture was incubated at 37°C for 10 min and the reaction was stopped by the addition of 50 μL of 20% trichloroacetic acid for PABA reaction or 100 μL of acetonitrile for 2-AF reaction. All experiments were run in triplicates. The amounts of acetylated product and that of the remaining nonacetylated substrate were determined by high pressure liquid chromatography. Each sample of the NAT reaction mixture was injected into the C18 reversed-phase column (Spherisorb 4.6 × 250 nm) of a Beckman HPLC (pump 168 and detector 126), and eluted at a flow rate of 1.2 ml/min. For AF and AAF, the solvent system was 20 mM KH_2PO_4 (pH 4.5)/ CH_3CN (53:47) with detection at 280 nm. The retention time was 6.5 min for AAF and 9 min for AF. For PABA and N-Ac-PABA, the solvent system was 50 mM acetic acid/CH_3CN (86:14) and detected was at 266 nm. The retention time

was 4.0 min for PABA and 6.5 min for N-Ac-PABA. All compounds were quantified by comparing the integrated area of the elution peak with that of the known amount of standards. NAT activity is expressed as nmol acetylated substrate per min per mg of cytosolic protein. The protein concentration of the tissue cytosol was determined by the method of Bradford [3], with bovine serum albumin as the standard. All samples were assayed in triplicates. Statistical analysis of the data was performed with an unpaired Student's t-test.

RESULTS

The aminofluorene-N-acetyltransferase (AF-NAT) activity and p-aminobenzoic acid-N-acetyltransferase (PABA-NAT) activity in rat glioma in vitro cultured cell cytosol are decreased by lomustine in this study. Activity of N-acetyltransferase (NAT) in rat glioma cell cytosol is found to be inhibited by lomustine. Its presence in the assay medium shows an inhibition in AF-NAT activity about of 17.6%, 31.5% and 45.4% in rat glioma in vitro cultured cells at 0.8 μM, 8 μM and 80 μM of lomustine concentrations respectively; while as PABA-NAT activity decreased about 10.4%, 27.1% and 45.8% in rat glioma in vitro cultured cells with 0.8 μM, 8 μM and 80 μM of lomustine concentrations respectively (Table 1).

Table 1. Effects of lomustine on NAT activity in cytosols of rat glioma cultured cell

Concentration of Lomustine	In cytosols	
	AAF (nmol/min/mg)	N-Ac-PABA (nmol/min/mg)
Control	1.08 ± 0.18 (1)	0.96 ± 0.16 (1)
0.8 μM	0.89 ± 0.18 (73.4%)	0.86 ± 0.16 (89.6%)
8 μM	[a]0.74 ± 0.14 (68.5%)	[a]0.70 ± 0.12 (63.9%)
80 μM	[b]0.59 ± 0.05 (45.4%)	[b]0.52 ± 0.08 (54.2%)

Values are mean ± S.D. n= 12

[a]differs between 8 μM lomustine and control. $p<0.05$

[b]differs between 80 μM lomustine and control. $p<0.01$

DISCUSSION

In mammals there are at least two arylamine N-acetyltransferase, NAT1 and NAT2, which show differences in substrate specificities. In mice, NAT1 does not catalyze the acetylation of PABA, but has activity for AF. Mouse NAT2 catalyzes acetylation of both PABA and AF [18]. In contrast, human NAT1 acetylates PABA and AF, but human NAT2 acetylates AF and not PABA [1]. NAT in human can be divided into slow and rapid acetylator phenotypes [21]. Variations in the activity, and affinity of the rapid and slow acetylator isoenzymes for aromatic amines substrates appear to account for the difference between the rapid and slow pathways of the in vivo acetylation. Interest in polymorphic acetylation of carcinogenic arylamines in human is largely due to the statistical linking of slow acetylator phenotypes with known occupational exposure to compounds which caused higher risk for bladder cancer [5,19], and rapid acetylator phenotypes to a higher risk for colorectal cancer [12,17]. N-acetyltransferase plays a key roles on the genotoxic effetcs of the heterocyclic aromatic amines. Metabolites of arylamine have been demonstrated to react with cellular DNA to form covalent DNA adducts, induce mutation and cause cancer in human [6,15,16]. Carcinogen-DNA adduct formation is an important step in chemical carcinogenesis [8,14]. Lomustine is commercially available nitrosourea derivative and chloroethyl nitrosourea compound which applied in anti-neoplastic therapy especially in central nervous system [2,4]. The present study indicates that the NAT activity is presented in rat glioma cell cytosol and lomustine could decrease NAT activity in rat glioma cytosol. But it is not known this NAT is NAT1 or NAT2, NAT belong to rapid or slow

acetylation. Further investigations is needed. The present studies demonstrated that lomustine can markedly decrease the NAT activity of rat glial tumor cells and the decreasing has a dose-dependent affect, the higher the concentration of lomustine, the higher the inhibition of NAT activity. Based on the results of present study it was clearly shown that lomustine did decrease NAT activity in rat glial tumor cells, which may offer some information about lomustine affects NAT activity, because NATs are able to metabolize arylamine compounds. Although it is not known whether the decrease or increase in NAT activity would led to decrease or increase tumor production or cancer prevention. But other investigators have already pointed out that elevated levels of NAT activity are associated with increased sensitivity to the mutagenic effects of many arylamines [7]. The attenuation of liver NAT activity is associated with several disease processes such as breast and bladder diseases [21]. Further investigations are needed. Many investigators have demonstrated that the combination of fast-acetylator phenotypes with a high consumption of meat increased the risk of colorectal cancer. The relation between meat intake, acetylator status, and colorectal carcinogenesis may involve exposure to heterocyclic amines that are generated by cooking meat for long periods at high temperatures [12,17]. But the development of glial tumor is still unknown. In other words, there is no any available information about NAT in normal glial cells because it is difficult to isolate normal glial cells from cortex tissue, this worths being studied by cell culture in future. In summary, the present studies show that lomustine induced inhibition of rat glial tumor in vitro cultured cells NAT activity.

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Lomustine 對大白鼠神經膠質瘤之體外培養細胞中乙醯轉移酶活性的影響

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目的：芳香胺化合物經 N 基乙醯轉移酶（N-acetyltransferase）之 N 基乙醯化可誘發癌，lomustine 是常用於治療腦瘤的一種抗癌物藥，本研究是 in vitro 探討 lomustine 對大白鼠神經膠質細胞瘤之體外培養細胞中乙醯轉移酶活性的影響。

材料與方法：取大白鼠神經膠質細胞瘤之體外培養細胞（n = 12）用乙醯化輔酶 A 作乙醯基而再利用高壓液相層析儀分析乙醯轉移酶的活性及 lomustine 對大白鼠神經膠質細胞瘤之體外培養細胞中乙醯轉移酶活性的影響，以 2-aminofluorene（2-AF）和 *p*-aminobenzoic acid（PABA）作受質。

結果：結果顯示大白鼠神經膠質細胞瘤之體外培養細胞對 AF 及 PABA 受質皆有乙醯轉移酶活性存在，其對 AF 及 PABA 的活性平均值各爲 1.08 ± 0.18 及 0.96 ± 0.16 nmol/min/mg protein 。在 0.8 μM, 8 μM and 80 μM 的 lomustine 濃度中，大白鼠神經膠質細胞瘤之體外培養細胞中 AF-NAT 活性降低約 17.6%, 31.5% and 45.4%, PABA-NAT 活性降低約 10.4%, 27.1% and 45.8% 。

結論： Lomustine 可降低大白鼠神經膠質細胞瘤之體外培養細胞中乙醯轉移酶的活性。

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關鍵詞：芳香胺化合物、 N 基乙醯轉移酶、神經膠質細胞瘤、 Lomustine